Remarks

The limitations of claim 31 have been incorporated into claim 30 and claim 31 has been cancelled. Claims 66-76 have been cancelled without prejudice or disclaimer.

Rejections under 35 U.S.C. 112, first paragraph. Claims 30-33 and 35-64 stand rejected under 35 U.S.C. 112 first paragraph as not enabling the use of any other bacteria besides *E. coli* and *Salmonella*. As applicants have argued in paper number 13, the instant invention encompasses a bacterial antigen delivery system comprising a novel environmentally limited viability system ("ELVS"). The ELVS comprises a gene essential for the viability of the bacterial antigen delivery system, wherein the essential gene is only *expressed* when the bacteria is in the host. The ELVS may also comprise a gene that is lethal to the bacterial antigen delivery system, wherein the lethal gene is only expressed when the bacteria is in the host.

Furthermore, the instant specification discloses (a) how to control the expression of an essential gene and a lethal gene, (b) how to select for and to clone an essential gene for any species of bacteria, and (d) specific examples of essential and lethal genes that may be used in the practice of the invention. Applicants submit that within the context of the state of the art at the time of filing, the skilled artisan would, without undue experimentation, have been able to practice the claimed methods utilizing methods known in the art, with a reasonable expectation of success. For example, on page 30, lines 2-9 of the instant specification, the inventors point out that "any bacteria in which an essential gene is known for which mutants can be made" can be used in the practice of the ELVS invention. The specification then goes on to describe (p. 30, lines 12-19) how to generate *asd* deletions in most *Enterobacteriaceae*. Further on (p. 30 line 20- p. 31 line 6), the specification describes how to screen for *asd-related* mutants in other types of bacteria. Furthermore, the skilled artisan at the time of filing would certainly know how to mutagenize any bacterial strain that can be grown in culture and to select for resulting auxotrophs (Curtiss, 1965), p. 30, lines 28-29 of the specification). Additionally, the specification lists several essential genes for both gram negative and gram positive bacteria on page 15, lines 19-22, which can be applied to the practice of this invention. Furthermore, the skilled microbiologist has the necessary skills to clone virtually any essential gene using straightforward gene complementation methods without undue experimentation. Thus, the specification in view of the highly developed state of the art of molecular microbiology provides sufficient guidance for the identification and isolation of an essential gene from any bacterial strain and the generation of strains harboring mutations in said essential gene in the practice of the invention as claimed.

The next crucial element to practicing the invention is that the essential gene must be under the control of an environmentally regulatable control sequence. It is important to note that this limitation was added to claim 30 to *clarify* that the essential gene is regulated by a promoter or regulator element, not by the selection of the plasmid harboring said essential gene through the provision of an exogenous agent to the bacteria. The specification fully describes temperature regulatable promoters and their use in controlling gene expression (page 20, line 22 through page 21, line 20) as well as methods of identifying temperature sensitive promoters using a cold-sensitive beta-galactosidase screening method, as described on page 21, lines 15-16. The specification further states on page 21, lines 19-20 that "[m]ethods for obtaining temperature-sensitive mutant repressors are well known" in the art. Once in hand, the skilled artisan is able to ligate the regulatable control element upstream of the essential gene, using common art recognized methods (*e.g.,* as described in Sambrook 1989 or other manuals described on page 23, lines 6-14.)

In a like manner the skilled artisan can place a lethal gene, whose expression results in bacterial cell death or lysis, under the control of an element that is controlled by a trans factor (which in this case is a repressor) that is expressed under the control of an environmentally regulatable control sequence. Again, the specification clearly and fully describes, in view of the advanced state-of-the-art and the high level of skill of the practicing artisan, how to do this. For example, lethal genes for many different types of bacteria, including both gram negative and gram positive, are provided on page 16, line 12 through page 17, line 6 of the specification. Those genes include *gef, hok, relF*, nucleases, phospholipases, endolysins and holins. The Patent Office, on page 6 under subheading number 19 of paper number 14, argues that P22 genes 13 and 19, which are examples of holins, would only operate in Salmonella or E. coli and that there are "no suggestions or teachings in the prior art to use any other host cells together with the Salmonella bacteriophage late genes." Applicants argue herein that the skilled artisan would readily accept that any holin or endolysin, for example, would have a reasonable chance of working the invention. For example, Krogh *et al.,* 1998, on page 2110, column 2, lines 3-14 explains that phages specific for both gram-positive and gram-negative bacteria encode holins and other peptidoglycan-degrading enzymes, which can be used in the practice of this invention. Young and Blasi (1995) state that the sequences of several Eubacteria phages reveal "11 or more unrelated gene families which share the functional and structural characteristics of holins" (abstract, lines 14-15). Therefore, it is apparent that the skilled artisan has full and sufficient guidance to produce an environmentally controlled lethal gene for any bacteria, given the advance state-of-the molecular microbiological arts and the information provided in the instant disclosure.

Applicants respectfully request, in view of the amendment to claim 30 and the arguments presented above, that the rejection of claims 30-33 and 35-64 be withdrawn.

Rejections under 35 U.S.C. 112, second paragraph. Claims 30-33 and 35-64 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and incomplete. The Patent Office points out that the method for inducing an immune response requires that antigens must be presented to the host. Pursuant to the recommendation of the Patent Office, claim 30 has been amended to include the step that the antigen is delivered to a cell of the host animal. Support for this amendment can be found on page 35, lines 16-22, page 36, lines 15-17, page 18, lines 21-25 and page 39, lines 6-9.

Claims 43, 44, 47, 51, 53-55 and 60 are rejected under 35 U.S.C. 112, second paragraph as being indefinite. Applicants respectfully point out that the alleged "abbreviations" are in fact the art recognized, *bona fide* names of the genes and thus are *not* abbreviations. While the Examiner points out that other documents use abbreviations similar to the gene names of the instant application, the skilled molecular microbiologist or immunologist to which the invention is directed would clearly recognize the italicized lower case gene names as actual genes, since these genes have been clearly identified in the literature and known for years in the art, and are clearly defined in the specification, which serves as the lexicon for the claims. The gene names are specifically and clearly identified and defined in the specification, as follows: *asd* is defined as a gene encoding a beta-aspartate semialdehyde dehydrogenase (specification at page 13, line 28); *dal* is a gene encoding a catalytic enzyme involved in the biosynthesis of diaminopimelic acid (specification at page 13, line 20); *dal* encodes an alanine racemase and *ddl* encodes a D-alanyl-D-alanine ligase (specification at page 15, lines 10-11); *fab, fad* and *pls* encode an enzyme involved in fatty acid biosynthesis, fatty acid degradation and phospholipid catalysis, respectively (specification at page 16, lines 2-3); *polA* encodes a DNA polymerase I (specification at page 23, lines 23-24); *virB* and *virF* are essential genes of *Shigella* and are described at least in Hromockyi (1992) or Tobe (1991) (specification at page 21, lines 3-6); *yopH* is described in Cornelis (1992) (specification at page 21, lines 9-12); *cI857* is defined as the temperature sensitive repressor of lambda P_L and lambda P_R (specification at page 22, lines 10-11); *P22 c2* is the P22 phage equivalent of lambda *cI* (specification at page 24, lines 8-9); *htrA* is defined at least in Johnson *et al* (1991) (specification at page 24, line 16); *cya* and *crp* are general control genes at least defined in Curtiss & Kelley (1987) (specification at page 34, lines 5-6); *PhoP* is defined at least in Miller (1989) and Galan (1989) (specification at page 34, line 6); *phoQ* is phenotypically equivalent to *phoP* and is defined at least in Miller (1989) (specification at page 34, line 17); *ompR* is defined at least in Dorman (1989) (specification at page 34, line 8); *galE* is involved in lipopolysaccharide ("LPS") synthesis and is defined at least in Germanier (1971, 1975) (specification at page 34, line 9); and *cdt* is at least defined in Kelley (1992) (specification at page 34, line 13). Given that the above mentioned gene names are not abbreviations but are the actual gene names recognized by the skilled microbiologist, and that the genes are either defined in the specification or at least referenced in

the literature, wherein the literature was available to the skilled artisan to which the invention is directed at the time of filing the instant application, applicants submit that the claims are definite and distinctly claim the invention. Applicants hereby request that the rejection against these claims be withdrawn.

Rejections under 35 U.S.C. 102. Claims 30-33, 35-38, 48-49 and 65 are rejected under 35 U.S.C. 102(b) as being anticipated by Nakayama *et al.* (1988), Curtiss *et al.* (1989), Jagusztyn-Krynicka (1993), Gentry-Weeks (1992), Schodel (1994) and/or Cieslak (1993), and under 35 U.S.C. 102(e) as being anticipated by Curtiss (patent '345). Those references teach an *asd* strain of bacteria harboring a plasmid that contains a functional copy of the *Asd* gene. This arrangement allows for the maintenance of the plasmid in the host bacterium *without* the provision of antibiotics or any other exogenous agent, as is the case when using antibiotic resistance or other selectable markers. It is important to note that the maintenance of the *Asd* plasmid of any and all of the applied references *do not depend on the provision of exogenous DAP.* In other words, DAP is not a substrate provided to maintain the plasmid in any of these references. Contrary to the assertion of the Examiner specifically on page 24, point number 63 of paper number 14, and generally throughout paper 14 from pages 7-25, if DAP were provided to the bacteria harboring the *Asd* gene, then there would be *no selective pressure to maintain the plasmid*, and the plasmid would be lost. Therefore, provision of DAP to the animal would lead to the loss of the plasmid, whereas lack of provision of DAP would allow the plasmid to be maintained. Hence the above cited references *do not* teach a method of environmental limitation of a bacterium, but merely a method of maintaining a plasmid without the use of antibiotics ("balanced-lethal system"). Applicants respectfully point out that the Patent Office has misapplied these references and has not adequately demonstrated that these references specifically teach that DAP or any other agent is provided to the host animal to maintain the viability of the bacteria while in the host and allow the bacteria to die when outside of the host. Applicants therefore request that the unsubstantiated and unsupported rejection of the claims under 35 U.S.C. 102(b), (e) be withdrawn and the claims allowed.

Rejections under 35 U.S.C. 103. Claims 30-33, 35-38, 50, 52-54 and 65 are rejected under 35 U.S.C. 103(a) for being obvious over Curtiss ('345) or Curtiss (1989) in view of Molin ('916). The Curtiss references teach complementation of an essential gene null mutation with a plasmid encoding said essential gene, whereby expression of the essential gene on the plasmid *is not under any environmental control* (see above discussion explaining that DAP is not provided to maintain the plasmid). Molin teaches the suppression of translation of a lethal gene under permissive conditions, whereby the production of said *ectopic* lethal gene occurs in the non-permissive environment. Given that "the critical limitation [of the instant claims] is expression of an *essential gene* [and not lack of expression of a lethal

protein] inside an animal and no expression outside of the animal, coupled with a gene that encodes for metabolism, growth,..." (paper number 14, page 14, point 37), the above references fail to teach this critical limitation.

However, in the interest of clarity, claim 30, and claims dependent therefrom, have been amended to include the limitations of a control sequence driving the expression of the essential gene in a permissive environment and the essential gene being a native gene wherein the chromosomal copy of the native gene is inactive. These amendments do not constitute new matter as they are based upon the detailed description in the specification. Mechanisms of controlling essential gene expression are discussed on pages 18-22 of the specification and the selection of an essential gene is discussed on pages 11-16. Given that Curtiss (1989 and '345) fail to teach regulatable control of expression of the essential gene and Molin fails to teach the use of a native essential gene in the restriction of growth of genetically engineered microbes, and there is no suggestion or motivation to combine these references existing in the art, the Patent Office has failed to establish a prima facie case of obviousness.

In light of the foregoing amendments and arguments, applicants respectfully request reconsideration of the rejection and withdrawal of these rejections.

The claims are rejected under 35 U.S.C. 103(a) as being unpatentable over Curtiss (1989 or '345) in view of Miller ('901). As stated above, the Curtiss references teach a method of maintaining a plasmid in a bacterium by complementation of an essential gene without supplying any exogenous substrate. Miller teaches the environmental regulation of expression of an heterologous antigen in a bacteria, which addresses an entirely different problem than environmental restriction of a recombinant bacteria. Given that neither of the Curtiss references nor the Miller reference teach the regulation of expression of an essential gene using a regulatable control sequence to restrict the viability of a bacterium and as all of the elements of the instant claims are not taught by the combination of the cited references, and because there is no motivation or suggestion to combine the teachings of the cited references since they are directed to solving different problems, the requirements for prima facie obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration of the rejection and withdrawal of these rejections.

Claims 30-33, 35-38, 47-49 and 65 are rejected under 35 U.S.C. §103(a) as being unpatentable over either Curtiss ('345 patent) or Curtiss (1989) in view of Curtiss (U.S. Patent No. 4,968,619). Applicants reiterate the arguments presented in paper number 13 dated March 12, 2001. Curtiss '345 and 1989 are cited as teaching a method of environmental regulation of an essential gene, while Curtiss ('619) is cited as teaching the use of an environmentally controlled promoter to limit the expression of a heterologous antigen, such that it is expressed in permissive environments (i.e. inside of an animal) and is not expressed in non-permissive environments (i.e. at room temperature, such as during preparation or

storage of the vaccine). However, as discussed above, Curtiss ('345 or 1989) teach the use of an essential gene to stabilize the vector in a population of host cells, wherein the essential gene is not under any *external* or *environmental* selective pressure. Furthermore, there is no teaching in any of the cited references of the use of an environmentally controlled promoter linked to the essential gene, as is the case in the claims as amended. In all of those references, the essential gene is expressed regardless of the environment that the host cell is in. There is no suggestion or motivation to use such regulation, because the function of those systems, *i.e.,* the balance-lethal plasmid maintenance and regulated expression of an antigen, would not be improved by regulation of the essential gene. As all of the elements of the instant claims are not taught by the cited references, nor is there a suggestion or motivation to combine their teachings, the requirements for a prima facie finding of obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38, 50, 52, 53 and 65 are rejected under 35 U.S.C. §103(a) as being unpatentable over Curtiss III ('345) in view of Molin et al. ('916) and Hershberger et al. Applicants present the same arguments as in paper 13, and emphasize for the record that the Examiner's position, as stated on page 20, point number 45 of paper number 14, is erroneous. The Curtiss references *do not* teach that the "host cell is attenuated and maintained through the environmental supplementation with DAP." If DAP were supplied to the host, then there would be no selective pressure to maintain the plasmid that comprises the functional essential gene and antigen. Hence, the position of the Examiner is in direct opposition to the teaching of these references. Curtiss III and Molin are cited as above. Hershberger is cited as showing an extra chromosomal vector that comprises a cI857 repressor for the purpose of stabilizing and selecting recombinant DNA host cells through the use of a lethal marker and complementary cloning vectors. As with the Curtiss III reference discussed above, Hershberger is directed to a method of stabilizing a recombinant vector in a population of host cells. The specification of that patent teaches that a cell constructed with a lethal marker on a chromosome, and a repressor on a recombinant vector, will die if it loses the vector. This is essentially a balanced-lethal system, which has no utility whatsoever for biological containment of the host cell. The host cell taught by that reference would survive in any environment that the wild-type cell would survive in, as long as the cell retained the recombinant vector comprising a repressor. Applicants point out that the claims as amended herein require that the bacterial cell comprise an environmentally limited viability system comprised of an essential gene that is a copy of a native gene, or a gene that is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell, wherein the essential gene is expressed when the cell is in the permissive environment (in the animal) and not expressed when the cell is in a non-permissive environment (outside of the animal). None of the cited references teach this limitation. The

systems of Hershberger and Curtiss III would not benefit from controlled expression of the gene, because the systems function optimally when the gene is expressed under all environmental conditions. Molin is discussed above, and the arguments provided there are applicable here as well. Molin does not teach regulated expression of an essential gene wherein the essential gene is a copy of a native gene. There would be no motivation to combine the teachings, because a balanced lethal system for retention of a vector in a population of host cells would not benefit from controlled expression of the essential gene. Likewise, the system of Molin would not benefit from the use of an essential gene that is a copy of a native gene because the use of an essential gene in that context is as a means of regulating the cell killing function of a lethal gene. None of the references disclose a native essential gene, which is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell, and is under the control of an environmentally regulated expression mechanism. As all of the elements of the claims are not taught by the cited references, and because there is no suggestion or motivation to combine the references, the requirements for establishing prima facie obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of these rejections.

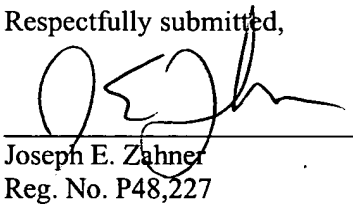
Claims 30, 33, 36-44, 46 and 52 are rejected under 35 U.S.C. §103(a) as being unpatentable over Curtiss III (U.S. Patent No. 5,840,483) in view of Youderian (1980). Curtiss III ('483 is cited as teaching a bacterial cell that is deficient in *asd* and comprises P22 bacteriophage genes, along with an extrachromosomal copy of the *asd* gene. Youderian is cited as teaching that genes 13 and 19 of P22 encode lysogenic products. Applicants present the same arguments as in paper 13, and emphasize for the record that the Examiner's position, as stated on page 24, point number 63 of paper number 14, is erroneous. The Curtiss references *do not* teach that "through supplementation of the environment inside the animal with DAP, the extrachromosomal vector carrying the Asd gene is stabilized in the population of host cell." Applicants argue, if DAP were supplied to the host, then there would be no selective pressure to maintain the plasmid that comprises the functional essential gene and other genes-of-interest. Therefore, the position of the Examiner is in direct opposition to the teaching of these references.

Curtiss III ('483) discloses a balanced lethal system, as do the other Curtiss III references, directed at stabilizing a host vector in a host cell population. In such a system, and as argued throughout these remarks, there is no environmental control mechanism associated with the *asd* gene. The function of the system described therein does not require, nor would it benefit from, such a control mechanism. The specific strain cited, λ3115, does not comprise a control mechanism whereby *asd* is expressed in a permissive environment and is not expressed in a non-permissive environment, as is required by the instant claims. The microbial cell disclosed comprises an extrachromosomal gene that encodes *asd*. Thus, the extrachromosomal vector carrying the *asd* gene is stabilized in the population of host cells

because without that vector, the product of the *asd* gene, which is essential for cell viability, is absent. This is true whether the host cell is inside of the animal or outside of the animal, because there is no control mechanism associated with the extrachromosomal *asd* gene. Furthermore, if DAP were provided to the host animal, the episome comprising *asd* and the antigen would be lost, given that the selective pressure would be removed. It is important to note that in the absence of exogenous DAP, the episome comprising *asd* and the antigen would be selected for and thus expression of the essential gene would occur outside of the host animal. Thus, all of the elements of the claims as amended are not disclosed in the cited references. In addition, there is no suggestion to combine the cited combination because the system of Curtiss III (a balanced-lethal system) does not benefit from controlled expression of the *asd* gene. Thus, the requirements for establishing prima facie obviousness have not been met. Applicants therefore respectfully request reconsideration and withdrawal of these rejections.

Conclusions: The claims as amended are in condition for allowance. In light of the above discussion, applicants respectfully request such action. If any issues remain, applicants request that the undersigned be contacted.

Respectfully submitted,



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August 6, 2001